IDEA

Declaration

I, the undersigned Professor Gregor Cevc, Ph.D. herewith declare as follows:

1. I am the named inventor of the US patent application No. 09/887,493, and I am the Chief Executive Officer of IDEA AG, Germany, assignee of the above-referenced patent application.

2. I have read and understood the Office Action dated 2 February 2005, in which the Patent Examiner rejected the presently pending claims of this application as being not patentable over DE 4 447 287 C1 in view of US 5,322,685 and Cevc et al, (J. controlled Release, 7 April 1997, 45, 211-226).

3. The Examiner stated that the present invention would not involve an inventive step as DE '287 teaches using BHT and benzyl alcohol, and thus teaches using antioxidants and microbicides. It is further mentioned that DE '287 does not specifically teach using these ingredients in amounts to yield the claimed effects. However, the opinion is held that a person of ordinary skill in the art would routinely optimize the amount of these ingredients to yield the most stable product, which would reasonably lead to a product having the claimed effects.

4. I herewith respectfully state that the above assessment does not take into consideration the essential point and thus an essential ingredient of the present invention. This statement is explained and substantiated as follows.

5. Suspensions of ultradeformable vesicles, Transfersomes®, which are described in the present patent application as well as in DE '287 are very special and delicate, novel drug carrier system. Unlike previously known carriers, Transfersomes® must be prepared from a finely balanced mixture of amphipats in which even a minor change in composition is likely to hamper the resulting carrier's ability to cross a semi-permeable barrier, such as the skin: incorporation of too much of an antioxidant or the choice of an inappropriate antioxidant will unacceptably diminish such mixed aggregate ("Transfersome®") deformability or stability. An antioxidant may, for example, transform the highly adaptable Transfersome® into a relatively stiff particle which will then clog the pores in a semi-permeable barrier through which Transfersomes® normally should or could pass;alternatively, an antioxidant may soften Transfersome® membrane too much, resulting in unstable vesicles that will break before or at least during pore passage. All this is unacceptable in drug delivery.

For example, butylated hydroxyanisol (BHA) or butylated hydroxytoluene (BHT), two very popular anti-oxidants, both tend to stiffen lipid vesicles, to a substantial albeit different extent, or else precipitate from vesicles suspension, either alone or as co-

precipitate with other components of the formulation. Our experiments specifically revealed that BHA incorporation into lipid vesicles in a conventional concentration range, achieved by adding BHA into the originally BHA-free and highly adaptable mixed amphipat aggregates, increases by more than 100% the likelihood of narrow pores clogging by such BHA containing aggregates or a sub-population of BHA-rich particles. In parallel, the adaptability of BHA-containing mixed membrane vesicles is lowered by approx. 40%, which corroborates the statement made in previous paragraph. Such precipitation is specifically illustrated by the attached experimental results (cf. comparative test 2), in which BHT was incorporated into a formulation in the commonly used and recommended amount for dermal preparations and which, due to the concomitant precipitation, is clearly unsuitable to the use as Transfersomes®. It is therefore essential to select the right relative amount of BHA/BHT and/or other membrane additives/softeners such that will keep pore clogging propensity low and carrier deformability high, thus preserving carrier functionality. It is equally important not to impair the formulations functionality by precipitation or co-precipitation of solid particles, such that would clog narrow pores in semi-permeable barriers (e.g. the skin). The present patent application, the know-how cited therein, and the given examples indicate how this goal can be achieved.

6. Similar conclusions and claims relate to the use of microbicides in the inventive formulations. A good example is phenoxyethanol, which is frequently used in the range of up to 5 percent to stabilise pharmaceutical formulations. In combination with Transfersomes® this microbicide cannot be used without scrutinising each individual formulation and its detailed properties. Specifically, incorporation of 0.5%, 1%, or 2% phenoxyethanol into otherwise functional Transfersomes® in a 2% suspension is tolerable, from the suspension homogeneity standpoint. When phenoxyethanol concentration is increased to 3% in such a suspension, however, phase separation sets-in and optically visible stripes / threads appear; the latter are diagnostic of non-vesicular structures that can not overcome narrow pores with the ease of Transfersomes®. An increase in phenoxyethanol concentration beyond 3%, which is in the upper range of the commonly used phenoxyethanol concentrations in pharmaceutical products, causes partial lipid solubilisation and the formation of three distinct sub-phases, only one of which is turbid and thus contains large aggregates / vesicles; the other two sub-phases contain solubilised lipid. Even the remaining vesicles also not properly functional, as they undergo unacceptably strong fragmentation during or after crossing of semi-permeable pores. Such vesicles are thus less suitable trans-barrier carriers as proper Transfersomes®, which remain essentially intact after pore crossing. Our observations made for the 10% lipid suspensions containing phenoxyethanol qualitatively similar. This suggests that absolute as well as relative concentration of

an additive to Transfersome® suspension both play a key role, which was not elaborated upon or disclosed in previous publications.

Irgasan is another commercially available antioxidant that is typically used in 0.1-0.3% concentration range in the water-based preparations. Incorporating this additive in a 2% suspension of functional Transfersomes® in 0.1-0.3% concentration range leads to Irgasan precipitation. The result are non-functional formulations, contaminated with the stiff Irgasan particles that clog narrow pores in semi-permeable barriers.

All above mentioned problems are solvable by applying the selection rules disclosed in the present patent application (high aggregate deformability and stability combined with a moderate increase in oxidation index, to be determined both on a case by case basis, as is described in the specification). Thus, using such rules in each individual case can lead to functionally adequate formulations.

The conclusion holds true for then water soluble as well as lipophilic microbicides, their critical or suitable relative and absolute concentrations co-depending on molecular properties of all other selected system ingredients. Lipophilic or amphiphilic microbicides, in particular, have a tendency to stiffen the mixed lipid bilayers that form Transfersomes®. Such additives even may form micro-crystals in vesicle suspension. The problem is often eminent below the range of concentrations in which macroscopic destabilisation/solubilisation is observed as well. In contrast, better water soluble microbicides have a relatively greater tendency to destabilise Transfersome® membranes in an unacceptable fashion.

Membrane stiffening or destabilisation makes vesicle suspensions less capable / incapable of passing through narrow pores with diameter smaller than the average vesicle size.

Furthermore, also in the case of microbicides, these substances very often underly precipitation or co-precipitation together with other ingredients of the formulation when thy are employed in the commonly recommended amount, as illustrated by the comparative example 1 relating to such addition of propylparabene to vesicle suspension.. The data clearly show that proper selection of additives and their amounts, also in the case of microbicides, is not a trivial exercise when making Transfersomes®.

7. DE '287 describes exclusively Transfersome® preparations from their basic composition standpoint. This document thus only recites general components that

could be used in combination with ultradeformable vesicles, but provides no teaching with regard to the addition of the specific suitable antioxidants or microbicides, nor does it offer a solution to the above mentioned problems associated with using such additives. The same applies to Cevc et al., which only describes Transfersomes®, and corticosteroids as their active agent, but does not mention the necessity to add antioxidants or microbicides to the formulation. Conversely, both cited references disclose that it is non-trivial to develop and obtain a functional carrier system. It is even more complicated to select most suitable further components, and their respective amounts, such that will maintain vesicle ultradeformability and thus allow transport of the carrier-associated active agents through narrow pores in a semi-permeable barrier, without modifying the key Transfersome® properties. The reason is that any added substance may directly affect the given Transfersome® properties after it gets incorporated into its membrane. If nothing major changes, such an addition is likely to modify the finely tuned hydrophilic/hydrophobic balance at the Transfersome® membrane-solution interface, which is not obvious and to the best of my knowledge was not addressed in any document published before the filing of the present patent. One possible reason is the complexity of underlying interactions, which may involve hydrogen bonding and electrostatic forces, hydrophobic interactions, (quasi)elastic strains within individual molecules, etc..

8. It is therefore not obvious which of the commonly used excipients could or should be added to a given Transfersome® system to maintain, rather than compromise, its essential characteristics. The situation is thus unlike that encountered with the previously known and simpler carrier systems, such as liposomes, that are far less delicate with regard to choosing the right membrane composition. Such classical carriers are therefore less influenced by addition of the commonly used microbicides or antioxidants, but also of no real usefulness for making extrapolations to Transfersomes®.

9. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true. I further witness that these statements were made with the knowledge that willful false statements and the like so made are punishable and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Munich, 19-07-05 .....
Date

(Prof. Gregor Cevc)

Confidential

Memorandum

IDEA AG

Comparative Tests - US Patent Application No. 09/887,493 based on PCT/EP98/08421

DATE: 11 July 2005

Subject-matter:

The following exemplary comparative tests were carried out in order to show that the proper selection of antioxidants and microbicides and corresponding amounts for the use in Transfersome® preparations is non-trivial, i.e. involves an inventive step. The experiments were performed with commonly used antioxidants and microbicides incorporated in Transfersome® preparations in the concentration range usually employed in the pharmaceutical industry.

Basic Formulation

The experiments are based on a typical Transfersome® preparation (e.g. according to DE 44 47 287 C1) additionally containing a corticosteroid, having the following composition:

Substance	Content [mg/g]
Prednicarbate	1.50
Soy phosphatidyl choline (SPC)	64.52
Tween 80	35.48
$Na_2HPO_4 \times 12\ H_2O$	7.72
$NaH_2PO_4 \times 2\ H_2O$	4.44
Glycerol	30.00
Water	856,34

Comparative tests

Test 1

A commonly used preservative, propylparabene, was added to the formulation as described, to reach its final amount of 0.8 weight-% (the usual concentration is about 0.9 weight-%). After the addition and mixing, at first streaks were observed and then a fine precipitate formed. This is due to the fact that either propylparabene precipitates from the suspension or else causes precipitation of another (co)precipitate. Anyhow, propylparabene in the commonly used concentration is not compatible with the described Transfersome® preparation.

Test 2

A commonly used antioxidant, butylhydroxytoluol (BHT), was added to the basic formulation as described, to reach its final amount of 0.25 weight-% (the usual concentration range is about 0.5 weight-%). Precipitation was observed, which verifies that BHT in the commonly used concentration range is incompatible with the described typical Transfersome® preparation.

Conclusion

These tests clearly show that due to the special composition of Transfersomes providing same with ultra-deformability and the capability of penetrating pores in a barrier, even if the diameter of the pores is smaller than the average penetrant diameter, the selection of useful antioxidants and microbicides ensuring sufficient stability without negatively influencing the deformability properties, is not trivial.

The common rules for selecting said substances and the commonly suggested amounts thereof are not generally applicable to such systems, as they have not been described before with respect to their stabilization.

Also, the cited references, i.e. DE 44 47 287 C1, US 5,322,685 and Cevc et al (Journal of Controlled Release (1997), vol. 45, pp. 211-226) do not provide any suggestion with respect to a proper selection of antioxidants or microbicides, but only generally mention the use thereof or do not mention them at all. There is no teaching regarding which substances or amounts are to be applied without impairing the Transfersomes' characteristic properties.